Effectiveness of Sanitizing Agents in Inactivating *Escherichia coli* in Golden Delicious Apples

G.M. Sapers, R.L. Miller, and A.M. Mattrazzo

ABSTRACT

Research was undertaken to develop improved methods of sanitizing apples contaminated with *Escherichia coli*. Unwaxed Golden Delicious apples, inoculated with nonpathogenic *E. coli*, were washed with 200 ppm Cl_2, commercial washing formulations, 5% H_2O_2, or combinations of H_2O_2 with commercial formulations at ca. 20°C or 50°C. Heated commercial formulations achieved a 2.5 log reduction in *E. coli* load, compared to a 2 log reduction for 200 ppm Cl_2. However, heated combinations of H_2O_2 with acidic surfactants achieved a 3–4 log reduction. Residual H_2O_2 in treated apples dissipated within several hours. These results demonstrate the efficacy of H_2O_2 in decontaminating apples containing *E. coli*.

Key Words: *Escherichia coli*, apples, decontamination, sanitizers, hydrogen peroxide

INTRODUCTION

OUTBREAKS OF DIARRHEA AND HEMOLYTIC UREMIC SYNDROME, associated with *Escherichia coli* O157:H7 in unpasteurized apple juice (Besser et al., 1993; Anon., 1996; CDC, 1997), have raised concerns about the adequacy of some sanitation practices and need for regulatory action. While the source of *E. coli* O157:H7 in such outbreaks has not been demonstrated, contamination of the fruit with animal feces has been suspected. Goverd et al. (1979) demonstrated an association between presence of manure in orchards and *E. coli* (presumptive) in juice prepared from "drops" (apples on ground). Deer, frequently observed in orchards in many apple-producing regions of the U.S., are known sources of *E. coli* O157:H7 (Rice et al., 1995). *E. coli* O157:H7 also has been isolated from seagull droppings. Fecal matter from such sources might contaminate apples directly or indirectly via spray irrigation water or windblown dust.

McLellan and Splittstoesser (1996) recommended that apples for cider production be brush-washed and rinsed prior to visual inspection. Several products for washing fruits and vegetables have been marketed, but little is known about their anti-bacterial action on plant surfaces. Information derived from animal carcass decontamination studies may be instructive, however. Various anti-microbial washes including hot water, chlorine, organic acids, and sodium hydroxide have been developed for microbial decontamination of animal carcasses (Dickson and Anderson, 1992). A 1% solution of trisodium phosphate (pH 11.6) was highly effective in killing planktonic and biofilm cells (on stainless steel chips) of *E. coli* O157:H7 (Somers et al., 1994). Trisodium phosphate rinses were effective in reducing the level of surviving *E. coli* O157:H7 on beef adipose but not tenderloin surface tissues following refrigerated storage (Fratamico et al., 1996). However, the possibility of induced alkali tolerance in *E. coli* O157:H7 associated with cider outbreaks should be investigated before such treatment could be applied with confidence to cider apples (Rowbury et al., 1996). Hot acid sprays and rinses were ineffective in decontaminating *E. coli* O157:H7 on beef surfaces (Brackett et al., 1994; Fratamico et al., 1996). Information on the efficacy of sanitizing agents in disinfecting apples is limited but suggests that conventional washing practices using chlorine and brushing may be ineffective (Hankinson, 1997). We have previously reported that 5% hydrogen peroxide was highly effective as a sanitizing wash for fresh mushrooms (Sapers et al., 1994) and had potential application in extending the shelf-life of other minimally processed fruits and vegetables (Sapers and Simmons, 1998). The objective of this study was to develop improved methods of sanitizing external surfaces of cider apples that would be effective in killing or removing *E. coli* O157:H7.

Authors Sapers, Miller and Mattrazzo are with the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038.

MATERIALS & METHODS

Preparation and inoculation of apples

Unwaxed Golden Delicious apples of known origin were obtained from produce distributors and stored at 4°C until needed. In most washing trials, apples were cut in half along the core axis prior to inoculation to simulate bacterial contamination of fragments of partially decayed apples exiting a washer/scrubber. In several trials, contaminated skin punctures were simulated by puncturing whole apples 4 times (stem end, calyx end, opposite sides at equator) with a 6.5 mm diameter nail to produce holes 1 cm deep. An inoculum containing non-pathogenic *E. coli* (ATCC 25922) was prepared by streaking a stock culture on Tryptic Soy Agar, (Difco, Detroit, MI), incubating for 24 h at 37°C, transferring the culture to 100 mL sterile H_2O by loop until the absorbance was 0.7 as measured at 590 nm with a Spectronic 21 spectrophotometer, and adding this concentrated inoculum to 3 L sterile distilled H_2O. The concentration of the diluted inoculum was determined by serial dilution with 0.1% peptone (Difco) and plating on Brain Heart Infusion Agar (BHIA, Difco) to be ~1.3×10^7 CFU/mL. Colonies were counted with a Bel Art Products (Pequannock, NJ) colony counter. Sets of 9 whole apples (or 18 halves) were immersed in the inoculum for 5 min, drained, and equilibrated in air beneath a plastic tub at ambient temperature for 30 min.

In washing trials, a series of commercial washing formulations for fruits and vegetables (Table 1) and their combinations with 5% H_2O_2 (Fisher Scientific, Fairlawn, NJ) were compared with 200 ppm Cl_2 (5.25% sodium hypochlorite (Chlorox Co., Oakland, CA), diluted

Table 1—Characteristics of commercial sanitizing washes for apples

Code	Composition	Concentration[a] (%)	pH
A	Acid anionic surfactant	1	2.4
B	Acid soap	5	3.4
C	Phosphoric acid + surfactant	1	2.1
D	Phosphoric acid + surfactant	1–2	1.7–1.8
E	Phosphoric acid + surfactant	1.6	1.9
F	Citric acid + surfactant	3.2	2.3
G	NaOH + surfactant	0.66–1	11.9–12.2
H	Trisodium phosphate	1–8	11.8–12.4
I	Surfactant	1	9.3
J	Peracetic acid + H_2O_2 + acetic acid	0.01–0.1	3.3–3.9

[a]Prepared by dilution of liquid concentrates or solid products supplied by their manufacturers.

1:125 and adjusted to pH 6.4 with citric acid). The commercial products were proprietary formulations, in most cases containing unidentified surfactants at unspecified concentrations. The range of use levels was intended to bracket manufacturer recommendations. Inoculated sets of whole apples or apple halves were decontaminated by washing in 4 L of sanitizer solution at ambient temperature, 50°C or 60°C on a shaker for 1 min. After treatment, the apple halves were drained, homogenized with 2 L sterile 0.1% peptone (Difco) for 1 min in a 3.78 L stainless steel blending container, and diluted with 0.1% peptone for surface plating on Petrifilm *E. coli* Count Plates (3M Microbiology Products, St. Paul, MN) or BHIA. Colonies were counted after 24 h at 37°C.

Additional washing trials were carried out with apples inoculated with *E. coli* ATCC 11775, *E. coli* ATCC 23716, and *Enterobacter aerogenes* B-199, all non-pathogenic strains, to examine variability in response to treatment.

Hydrogen peroxide residues in washed apples

To determine residual H_2O_2 concentrations in apples washed with H_2O_2 solutions, treated apples (whole or halves, rinsed in water or not rinsed) and untreated controls were homogenized with a Waring Blendor immediately following treatment and tested for peroxide by the Reflectoquant analysis system (EM Science, Gibbstown, NJ) at frequent intervals over several hours. Similar measurements were made on juice prepared from homogenates of treated apples by straining through cheesecloth.

Statistical analyses

Population reduction data were analyzed for differences in response to treatments by ANOVA, t-tests and the Bonferroni LSD test to separate means (Miller, 1981). All statistical analyses were performed with *SAS/STAT software* (SAS Institute Inc., Cary, NC).

RESULTS & DISCUSSION

Attachment of *E. coli* to apple surfaces

In a study of *E. coli* attachment to apple surfaces, we compared whole, punctured and half Golden Delicious apples (unwaxed) that had been inoculated with ATCC 25922 (Table 2). Counts were lower in inoculated whole apples than in inoculated punctured or half apples. It appeared, therefore, that *E. coli* bound to or was entrapped on the cut surface of apples to a greater extent than on the intact skin. These results suggested that apples containing punctures or cuts or that had been broken into pieces by scrubbing might become more heavily contaminated with *E. coli* than intact, unblemished apples if immersed in contaminated water. Such contamination might occur in a wet dump tank or flume if the water were not adequately chlorinated.

In these experiments, *E. coli* populations in inocula were adjusted to produce counts in inoculated apples (halves or punctured) exceeding 10^5 CFU/g. Such counts would permit direct measurement of population reductions obtained by anti-microbial washes as great as 5 logs. In contrast, uninoculated Golden Delicious apples showed no *E. coli* colonies on Petrifilm (<2 CFU/g) and had total plate counts on BHIA between 10^2 and 10^3 CFU/g (mean=5.7×10^2 CFU/g). Since *E. coli* O157:H7 population levels in naturally contaminated apples were not known, population reductions obtained by the treatments described herein may or may not be sufficient to assure product safety. However, by comparing these population reductions with that obtained with 200 ppm Cl_2 (pH 6.4), the relative treatment efficacy could be inferred.

Effects of chlorine and commercial sanitizer washes

Preliminary studies suggested that a 1 min wash with water at room temperature reduced the load of *E. coli* (ATCC 25922) adhering to exposed apple flesh by <1 log (data not shown). Washing uncut inoculated apples with 200 ppm Cl_2 (pH 6.4) also reduced the *E. coli* population by <1 log (Table 3). With inoculated half apples, log reductions for this treatment were between 1.4 and 2.0 for three *E. coli* strains and one *E. aerogenes* strain.

Table 2—Attachment of *E. coli* (ATCC 25922) to skin, punctures, and cut surface of Golden Delicious apples

Sample[a]	Inoculum $\log_{10}$(CFU/mL)[b]	Apple homogenate $\log_{10}$(CFU/g)[b,c]
Whole apples	7.1±0.1	4.3[e]
Punctured apples	7.2±0.1	5.5[d]
Apples cut in half	7.2±0.1	5.4[d]

[a]Nine apples, either whole, punctured four times (stem end, calyx end, opposite sides at equator) with a nail to produce holes 1 cm deep and 6.5 mm in dia or cut in half at equator; immersed in 3 L inoculum with agitation for 5 min; air-dried 30 min.
[b]Means of six trials ± SD; data from BHIA plate counts.
[c]Mean $\log_{10}$ CFU/g for uninoculated controls was 2.7±0.3; *E. coli* not detected on Petrifilm (<2 CFU/g).
[d-e]Means in same column with no letter in common were different (P<0.05) by the Bonferroni LSD mean separation test.

Table 3—Effect of 200 ppm Cl_2 (pH 6.4) wash on *E. coli* adhering to surface of inoculated Golden Delicious apples

Inoculum[a]	Sample[b]	No. of trials	$\log_{10}$ reduction[c]
E. coli ATCC 25922	Whole apples	2	0.5[f]
	Half apples	6	1.9[de]
E. coli ATCC 23716	Half apples	3	1.4[e]
E. coli ATCC 11775	Half apples	2	1.7[de]
Enterobacter aerogenes	Half apples	3	2.0[d]

[a]Inoculated by immersion for 5 min in 3 L diluted *E. coli* inoculum containing approx 2.0×10^7 CFU/mL.
[b]Apples washed for 1 min with agitation.
[c]Based on $\log_{10}$(CFU/g) of corresponding inoculated controls; data from BHIA plate counts.
[def]Means with no letter in common were different (P<0.05) by the Bonferroni LSD mean separation test.

In trials to determine the effects of commercial fruit and vegetable sanitizing agents on inoculated apple halves, most formulations were comparable to 200 ppm Cl_2 (pH 6.4), achieving reductions of ~2 logs (Table 4). These formulations represented three product types: acidic surfactants (Products A-F), alkaline combinations (G-I), and peracetic acid combinations (J). However, Product I, a slightly alkaline surfactant formulation, gave only a one log reduction. When the wash solutions were heated to 50°C, reductions of ~2.5 logs were obtained with Products D and J, a significant increase over the reduction at ambient temperature based on individual F-test contrasts.

Hydrogen peroxide as a sanitizing agent for apples

Inoculated apple halves washed with 5% hydrogen peroxide showed population reductions generally in the range of 3–4 logs (Table 5 and 6). Populations of endogenous bacteria (not *E.coli*) typically were reduced by ≥1 log to residual populations ≤10^2 CFU/g. BHIA was used in preference to Petrifilm to enumerate surviving *E. coli* in these trials since counts were higher with the former medium for samples treated with 5% H_2O_2 where the number of survivors was small and low dilution was required. This may have been due to injury to surviving *E. coli* induced by the washing treatments or to the presence of treatment residues in the lowest dilutions that inhibited bacterial growth. In all likelihood, variation within the 3–4 log reduction range was a reflection of the low counts after hydrogen peroxide treatments. Both media gave similar results with inoculated controls (indicating minimal contaminating background microflora) and apples treated with 200 ppm Cl_2 or 2.5% H_2O_2 (data not shown). Apples treated with 5% H_2O_2 at ambient temperature contained a small population of surviving *E. coli*, equivalent to a reduction of about 3.4 logs. Similar results were obtained with combinations of 5% H_2O_2 and commercial sanitizing agents tested previously. Population reductions after treatment with heated (50°C) 5% H_2O_2 and combinations of 5% H_2O_2 with 5% Sanitizer B, 1% Sanitizer C, 1–2% Sanitizer D, 1.6% Sanitizer E, or 3.2% Sanitizer F were not different from those obtained at ambient temperature (in the range of 3–4 logs). However, in most cases population reductions were slightly greater at the higher treatment temperature. Although the combination of H_2O_2 and 2% Sanitizer H (trisodium phosphate) was effective in decontaminating apples (data not shown), variable results were obtained with this treatment, especially at 50°C, due to the instability of alkaline H_2O_2 solution (rapid loss of H_2O_2 accompanied by gas evolution). Therefore,

Table 4—Effect of commercial sanitizing agents on *E. coli* (ATCC 25922) on inoculated Golden Delicious apple halves[a]

Treatment	No. of trials	Log_{10} reduction[b]
200 ppm Cl_2 (pH 6.4)	5	2.1[c]
1% Sanitizer A	2	1.9[c]
1% Sanitizer A at 50°C	2	2.1[c]
5% Sanitizer B at 50°C	2	2.0[c]
1% Sanitizer C	2	2.0[c]
1% Sanitizer C at 50°C	2	2.3[c]
1% Sanitizer D	3	1.9[c]
1% Sanitizer D at 50°C	2	2.6[c]
2% Sanitizer D at 50°C	3	2.3[c]
1.6% Sanitizer E	2	2.0[c]
3.2% Sanitizer F	2	2.1[c]
4% Sanitizer H	4	2.4[c]
4% Sanitizer H at 50°C	3	2.4[c]
1% Sanitizer I	2	1.0[d]
1000 ppm Sanitizer J	2	2.0[c]
1000 ppm Sanitizer J at 50°C	3	2.6[c]

[a]For each treatment, nine apples cut in half, inoculated by immersion for 5 min in 3 L diluted *E. coli* inoculum containing ≈2.0×10^7 CFU/mL, and washed 1 min.
[b]Based on log_{10}(CFU/g) of corresponding inoculated controls (mean=5.3±0.2); data from BHIA plate counts.
[c-d]Means with no letter in common were different (P<0.05) by the Bonferroni LSD mean separation test.

Table 5—Efficacy of washes containing hydrogen peroxide and commercial sanitizing agents on decontamination of Golden Delicious apple halves inoculated *E. coli* (ATCC 25922)[a]

Treatment[b]	No. of trials	Log_{10} reduction[b]
200 ppm Cl_2 (pH 6.4)	5	2.0[e]
2.5% H_2O_2	2	2.7[de]
5% H_2O_2	4	3.4[de]
5% H_2O_2 at 50°C	7	3.8[d]
5% H_2O_2 + 5% Sanitizer B at 50°C	2	>4.1[d]
5% H_2O_2 + 5% Sanitizer C at 50°C	2	>4.1[d]
5% H_2O_2 + 1% Sanitizer D	2	3.3[de]
5% H_2O_2 + 1% Sanitizer D at 50°C	2	4.2[d]
5% H_2O_2 + 2% Sanitizer D at 50°C	2	>4.1[d]
5% H_2O_2 + 1.6% Sanitizer E at 50°C	3	3.8[d]
5% H_2O_2 + 3.2% Sanitizer F at 50°C	2	3.6[de]
5% H_2O_2 + 1% Sanitizer I	2	3.2[de]
5% H_2O_2 + 1% Sanitizer I at 50°C	2	3.2[de]

[a]For each treatment, nine apples cut in half and inoculated by immersion for 5 min in 3 L diluted *E. coli* inoculum containing ≈1.3×10^7 CFU/mL.
[b]1 min wash.
[c]Based on log_{10}(CFU/g) of corresponding inoculated controls and treatments; data from BHIA plate counts.
[d-e]Means with no letter in common were different (P<0.05) by the Bonferroni LSD mean separation test.

this treatment would not be advisable for use in sanitizing apples.

Data cited by Block (1991), indicated that *E. coli* was relatively sensitive to treatment with 3% H_2O_2; the D value was 0.57 min, compared to 1.04 min for *Bacillus cereus*, 2.35 min for *Staphylococcus aureus*, and 8.55 min for *Aspergillus niger*. Destruction of bacterial spores increased with increasing peroxide concentration and treatment temperature. At the high H_2O_2 concentrations we employed, the lethal response of *E. coli* did not require active cellular metabolism (Juven and Pierson, 1996). At the lower concentrations such as may occur briefly as residues following treatment, this bacterium lacks essential mechanisms for bacterial resistance to H_2O_2, i.e., glutathione peroxidase. Furthermore, the high diffusibility of exogenous H_2O_2 into the bacterial cell would exceed the capacity of endogenous catalase to decompose the peroxide.

Comparisons of washing treatments with 5% H_2O_2 applied at 60°C showed that treatment was slightly more effective than treatments applied at 50°C (Table 6). Combinations of 5% H_2O_2 with acidic surfactants (Sanitizers B and D) were comparable to 5% H_2O_2 alone.

Washing trials with apples inoculated with *E. aerogenes* and two other non-pathogenic *E. coli* strains (Table 7) showed variation in response to the washing treatments. Log reductions varied between 1.37 and 1.96 for 200 ppm Cl_2, between 2.37 and 3.86 for 5% H_2O_2 at 50°C, and between 2.72 and 4.16 for 5% H_2O_2 + 2% Sanitizer D at 50°C. Reductions for the last treatment were greater with ATCC

Table 6 — Effect of treatment temperature on decontamination of Golden Delicious apple halves inoculated with *E. coli* (ATCC 25922)[a]

		Log_{10} reduction[c]	
Treatment[b]	No. of Trials	50°C	60°C
5% H_2O_2	3	2.8[d]	3.3[e]
5% H_2O_2 + 5% Sanitizer B	3	3.0[d]	3.2[d]
5% H_2O_2 + 2% Sanitizer D	3	2.7[d]	3.1[d]

[a]For each treatment, nine apples cut in half and inoculated by immersion for 5 min in 3 L diluted *E. coli* inoculum containing ≈1.3×10^7 CFU/mL.
[b]1 min wash.
[c]Based on log_{10}(CFU/g) of corresponding inoculated controls; data from BHIA plate counts.
[d-e]Within the same row, means with no letter in common were different (P≤0.05) by the t-test.

Table 7—Variation in response of *E. coli* strains in inoculated Golden Delicious apples to decontamination treatments[a]

	Mean log_{10} reduction[c]			
Treatment	*E. coli* ATCC 25922	*E. coli* ATCC 23716	*E. coli* ATCC 11775	*Enterobacter aerogenes* B-199
200 ppm Cl_2 (pH 6.5)	1.8de	1.4e	1.7de	2.0[d]
5% H_2O_2 at 50°C	3.9[d]	2.4e	2.6de	3.3[de]
5% H_2O_2+2% Sanitizer D at 50°C	4.0[d]	2.7[e]	2.7[e]	4.2[d]
No. trials	6	3	2	3

[a]For each treatment, nine apples cut in half and inoculated by immersion for 5 min in 3 L diluted *E. coli* inoculum containing ≈1.3×10^7 CFU/mL.
[b]1 min wash.
[c]Based on log_{10}(CFU/g) of corresponding inoculated controls; data from BHIA plate counts.
[d-e]Within each row, means with no letter in common were different (P≤0.05) by the Bonferroni LSD mean separation test.

25922 and *E. aerogenes* than with the other strains. Further study is required to determine whether such variation would extend to *E. coli* O157:H7 and was due to differences in the acid tolerance of the bacterial strains (Miller and Kaspar, 1994) or to other factors.

Washing trials with cut apples represent a "worst case" scenario. Additional trials were run with inoculated whole apples to confirm the efficacy of hydrogen peroxide treatments under more typical conditions (Table 8). *E. coli* levels on these apples were about 1 log lower than population levels on inoculated apple halves (see Table 2). Both 5% H_2O_2 and the combination of 5% H_2O_2 with 1% Sanitizer C (each treatment applied at 50°C) reduced the *E. coli* population by more than 2.5 logs. Under the same conditions, treatment with 1% sanitizer C at 50°C yielded a reduction of only 1.5 logs, while treatment with 200 ppm Cl_2 (pH 6.4) yielded a reduction of <0.5 log (See Table 3). Treatment with water at 50°C resulted in a population reduction of only 1.2 logs, similar to that obtained with the sanitizer solution. In preliminary experiments with apple halves inoculated with *E. coli* O157:H7, treatment with water at 50°C reduced the population by only 0.5 log (Sapers and Buchanan, 1997). Therefore, the H_2O_2 treatments would be substantially more effective than conventional treatments with contaminated intact or cut apples.

Hydrogen peroxide residues in treated apples

Homogenates of half-apple samples treated with 5% H_2O_2 contained H_2O_2 residues in excess of 1000 ppm immediately following treatment which declined to relatively low levels (i.e., 20 ppm) over several hours (Table 9). H_2O_2 levels in juice prepared from these homogenates by straining through cheesecloth remained high, even after 4 h. Presumably, this was due to the removal of particulates to which endogenous catalase, the enzyme responsible for H_2O_2 degradation, was bound. H_2O_2 levels in treated apples halves could be reduced to <20 ppm immediately following treatment by rinsing with water.

Whole apples contained much lower H_2O_2 levels following treatment, i.e., <100 ppm, which in juice dissipated within several hours to background levels seen in untreated controls. Preliminary experiments demonstrated the presence of as much as 10 ppm endogenous

Table 8—Efficacy of washes containing hydrogen peroxide and commercial sanitizing agents on decontamination of uncut Golden Delicious apples inoculated with *E. coli* (ATCC 25922)[a]

Treatment	No. of trials	Log_{10} reduction
5% H_2O_2 at 50°C	2	2.7[d]
5% H_2O_2+1% Sanitizer C at 50°C	2	2.8[d]
1% Sanitizer C at 50°C	2	1.5[e]
H_2O at 50°C	2	1.2[e]

[a]For each treatment, nine whole apples inoculated by immersion for 5 min in 3 L diluted *E. coli* inoculum containing ≈1.3×10^7 CFU/mL.
[b]1 min wash.
[c]Based on log_{10}(CFU/g) of corresponding inoculated controls (mean = 4.2 ± 0.2); data from BHIA plate counts.
[d-e]Means with no letter in common were different (P≤ 0.05) by the Bonferroni LSD mean separation test.

Table 9—Hydrogen peroxide residues in Golden Delicious apples washed with 5% H_2O_2[a]

Sample	Rinse	Time after Treatment (min)	Residual H_2O_2 (ppm)[b] Homogenate	Juice
Half apples	No	5	1110	740
		120	240	660
		240	20	580
	Yes	5	18	—
Whole apples	No	8	—	72
		60	—	16
		120	—	4
		180	—	3

[a]1 min wash.
[b]Determined by Reflectoquant method.

H_2O_2 in homogenized apple tissue from untreated fruits (data not shown). This was probably a consequence of flavin-generated peroxide in the disrupted tissue (Fruton and Simmonds, 1953).

While H_2O_2 is classified as GRAS, its use in foods is limited to certain specified applications (CFR, 1994). The applications we describe would require regulatory approval by the Food & Drug Administration prior to use by apple packers or cider producers.

CONCLUSIONS

E. COLI POPULATIONS WERE GREATER ON CUT OR PUNCTURED SURFACES of fresh apples than on the intact skin. Chlorine solutions and several commercial sanitizing agents for fruits and vegetables achieved comparable population reductions in apples inoculated with *E. coli*. Slight increases in effectiveness were obtained by applying them at 50°C rather than at ambient temperature. However, much higher population reductions were obtained by washing with 5% H_2O_2 or combinations of H_2O_2 with commercial sanitizing agents, both heated to 50–60°C. Population reductions generally were within the range of 3–4 logs, and some strain-to-strain differences in response to treatment were found. H_2O_2 residues in juice from treated whole apples declined to low levels within several hours. Higher levels in treated cut apples could readily be removed by rinsing.

REFERENCES

Anonymous. 1996. *E. coli* causes apple juice product recall by California firm. Food Chemical News, Nov. 4, p. 44.

Besser, R.E., Lett, S.M., Weber, J.T., Doyle, M.P., Barrett, T. J., Wells, J.G., and Griffin, P.M. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *E. coli* O157:H7 in fresh-pressed apple cider. JAMA 269: 2217-2220.

Block, S.S. 1991. Peroxygen compounds. Ch. 9 in *Disinfection, Sterilization and Preservation*, 4th ed., S.S. Block (Ed.), p. 167-181. Lea & Febiger, Philadelphia.

Brackett, R.E., Hao, Y.-Y., and Doyle, M.P. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. J. Food Protection 57: 198-203.

CDC. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, Oct. 1996. MMWR 46: 4-8.

CFR. 1994. Hydrogen peroxide. Code of Federal Regulations 21, Parts 170-199, Section 184.1366, April 1, U.S. Govt. Print. Office, Washington, DC.

Dickson, J.S. and Anderson, M.A. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. J. Food Protection 55: 133-140.

Fratamico, P.M., Schultz, F.J., Benedict, R.C., Buchanan, R.L., and Cooke, P.H. 1996. Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. J. Food Protection 59: 453-459.

Fruton, J.S. and Simmonds, S. 1953. The flavin nucleotides and flavoproteins. Ch. 13 in *General Biochemistry*. John Wiley & Sons, Inc., New York.

Goverd, K.A., Beech, F.W., Hobbs, R.P., and Shannon, R. 1979. The occurrence and survival of coliforms and salmonellas in apple juice and cider. J. Appl. Bacteriol. 46: 521-530.

Hankinson, T.R. 1997. Personal communication. Pure Produce, Inc. Worcester, MA.

Juven, B.L. and Pierson, M.D. 1996. Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation. J. Food Protection 59: 1233-1241.

McLellan, M.R. and Splittsstoesser, D.F. 1996. Reducing risk of *E. coli* in apple cider. Food Technol. 50(12): 174.

Miller, L.G. and Kaspar, C.W. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Protection 57: 460-464.

Miller, R.G. Jr. 1981. In *Simultaneous Statistical Inference*, p. 67. Springer-Verlag, New York.

Rice, D.H., Hancock, D.D., and Besser, T.E. 1995. Verotoxigenic *E. coli* O157:H7 colonization of wild deer and range cattle. Vet. Rec. 137: 524.

Rowbury, R.J., Lazim, Z., and Goodson, M. 1996. Regulatory aspects of alkali tolerance induction in *Escherichia coli*. Letters in Appl. Microbiol. 22: 429-432.

Sapers, G.M. and Buchanan, R.L. 1997. Unpublished data. USDA-ARS, Eastern Regional Research Center, Wyndmoor, PA.

Sapers, G.M., Miller, R.L., Miller, F.C., Cooke, P.H., and Choi, S.-W. 1994. Enzymatic browning control in minimally processed mushrooms. J. Food Sci. 59: 1042-1047.

Sapers, G.M. and Simmons, G.F. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. Food Technol. 52(2): 48-52.

Somers, E.B., Schoeni, J.L., and Wong, A.C.L. 1994. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium*. Int. J. Food Microbiol. 22: 269-276.

Wallace, J.S., Cheasty, T., and Jones, K. 1997. Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. J. Appl. Microbiol. 82: 399-404.

Ms received 10/2/98; revised 2/17/99; accepted 3/10/99.

We acknowledge the technical contribution of M. Evans at the Eastern Regional Research Center. We thank Dr. John G. Phillips, Consulting Statistician, North Atlantic Area, ARS, for analysis of the data. We also thank the following companies that provided the washing formulations: Diversey Corp., DuBois Division, Cincinnati, OH; Ecolab, Food & Beverage Division, St. Paul, MN; Elf Atochem North America, Inc. (DECCO), Monrovia, CA; Alex C. Fergusson, Inc., Frazer, PA; Microcide, Inc., Detroit, MI; and Rhodia, Inc., Washington, PA.

Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.